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Phenotypic and Molecular Analysis of Tellurite Resistance among Enterohemorrhagic *Escherichia coli* O157:H7 and Sorbitol-Fermenting O157:NM Clinical Isolates

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A total of 66 (98.5%) of 67 enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strains had increased potassium tellurite (Te) MICs (32 to 1,024 μg/ml), grew on Te-containing media, and possessed Te resistance (*ter*) genes, whereas 83 (96.5%) of 86 sorbitol-fermenting (SF) EHEC O157:NM strains had Te MICs of ≤4 μg/ml, did not grow on Te-containing media, and lacked *ter* genes. Optimal detection of SF EHEC O157:NM strains requires Te-independent strategies.

Tellurite (Te)-resistant (Te^r) non-sorbitol-fermenting enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strains cause diarrhea and hemolytic-uremic syndrome (HUS) worldwide (17), but sorbitol-fermenting (SF) EHEC O157:NM (nonmotile) strains have emerged as pathogens only in Europe (1, 6, 8, 12) and Australia (3) so far. SF EHEC O157:NM strains are not distinguishable from commensal *E. coli* strains on sorbitol MacConkey agar (SMAC), and do not grow (11) on cefixime-Te (CT)-SMAC (22), which is frequently used for selective isolation of EHEC O157:H7 strains from feces, foods, and the environment (2, 4, 5, 9, 13, 20, 21). Te^r in EHEC O157:H7 is associated with the *ter* (*terZABCDEF*) gene cluster (19), duplicated in strain EDL933 in O islands OI 43 and OI 48 (14). One of these islands was originally identified in strain 86-24 (16) as the Te^r and adherence-conferring island (16). Te^r in SF EHEC O157:NM strains has not been investigated. Because Te susceptibility (Te^s) could thwart the detection of such strains on media containing Te, we investigated Te^r and the presence of *ter* genes in a large collection of SF EHEC O157:NM clinical isolates. We compared these characteristics with those of EHEC O157:H7.

Isolation and characterization of strains. A total of 67 EHEC O157:H7 and 86 SF EHEC O157:NM strains were isolated between 1987 and 2003 from patients with HUS (n = 118) or bloody (n = 11) or watery (n = 19) diarrhea and from asymptomatic carriers (n = 5). To avoid biases from strains selected by their Te^r, only EHEC O157 strains isolated on Te-free media by methods described previously (6, 10, 11) were included in this study, and they comprise a subset of the 572 *E. coli* O157 strains recovered during this interval. The 67 EHEC O157:H7 strains belonged to Shiga toxin (Stx) genotypes stx_1 (2 strains), stx_2 (28 strains), $stx_1 + stx_2$ (6 strains), stx_{2c} (8 strains), $stx_1 + stx_{2c}$ (4 strains), and $stx_2 + stx_{2c}$ (19 strains). All 86 SF EHEC O157:NM strains contained stx_2 only. Te MICs (the lowest Te concentrations which inhibited growth) were determined using a microdilution broth method (15). Each strain was tested in duplicate and in two independent experiments using 4×10^4 to 5×10^4 CFU/well and serial twofold concentrations (1 to 1,024 μg/ml) of potassium Te (Sigma, Taufkirchen, Germany) in 100 μl of Luria-Bertani (LB) broth. The ability of EHEC O157 to grow on solid media containing the Te concentration routinely used for *E. coli* O157:H7 selective isolation was tested by inoculating 10^5 CFU from overnight LB broth cultures on CT-SMAC (potassium Te 2.5, μg/ml; cefixime, 0.05 μg/ml [Oxoid, Basingstoke, United Kingdom]) and LB agar plus 2.5 μg of potassium Te per ml (LB-Te agar). The presence of *ter* genes was determined by PCRs using primer pairs TerZ1 plus TerZ2 (*terZ*), TerA1 plus TerA2 (*terA*), TerB1 plus TerB2 (*terB*), TerC1 plus TerC2 (*terC*), TerD1 plus TerD2 (*terD*), TerE1 plus TerE2 (*terE*), and TerF1 plus TerF2 (*terF*) (19), with strains EDL933 and C600 as positive and negative controls, respectively. Genomic DNA was digested (BamHI and PstI; New England Biolabs, Frankfurt, Germany), separated in 0.6% agarose, and probed under stringent conditions with digoxigenin-labeled *terC* (19) (DIG DNA labeling and detection kit; Roche Molecular Biochemicals, Mannheim, Germany).

Te^r and *ter* presence among EHEC O157. Of 67 EHEC O157:H7 strains, 50 (74.6%) and 16 (23.9%) had high (256 to 1,024 μg/ml) or intermediate (32 to 128 μg/ml) Te MICs, respectively (Table 1). All 66 Te^r strains grew well on CT-SMAC and LB-Te agar and contained *terZ, terA, terB, terC, terD*, *terE*, and *terF* (Table 1). One EHEC O157:H7 strain (5288/91) had a Te MIC of <1 μg/ml, failed to grow on CT-SMAC and LB-Te agar, and lacked all *ter* genes (Table 1). In contrast, 83 (96.5%) of 86 SF EHEC O157:NM strains were susceptible to Te (Te MICs of ≤4 μg/ml) (Table 1). Of these 83 strains, 70 (84.3%) failed to grow on CT-SMAC and LB-Te agar and 13 strains (15.7%) were strongly inhibited on both media (<10 colonies grew after overnight incubation). All 83 Te^s SF EHEC O157:NM strains lacked *ter* genes (Table 1).

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TABLE 1. Te^r and the presence of *ter* genes among EHEC O157:H7 and SF EHEC O157:NM strains

Serotype	Total no. of strains	SF[b]	No. (%) of strains with a characteristic									
			Te MIC (μg/ml) in range of:				Robust growth[c] on:		No or strongly reduced growth[d] on:		*ter* genes[e]	
			256–1024	32–128	2–4	<1	CT-SMAC	LB-Te agar	CT-SMAC	LB-Te agar	Present	Absent
O157:H7	67	0	50 (74.6)[f]	16 (23.9)[f]	0 (0)	1 (1.5)	66 (98.5)	66 (98.5)	1 (1.5)	1 (1.5)	66 (98.5)[g]	1 (1.5)
O157:NM[a]	86	86	1 (1.2)[f]	2 (2.3)[f]	40 (46.5)	43 (50.0)	3 (3.5)	3 (3.5)	83 (96.5)	83 (96.5)	2[h] (2.3)[g]	84[i] (97.7)

[a] NM, nonmotile.
[b] SF, sorbitol fermentation as determined after overnight incubation on SMAC agar.
[c] Robust growth, >1,000 colonies of normal size per plate.
[d] No growth, no colonies grown; strongly reduced growth, <10 colonies reduced in size present after overnight incubation.
[e] As determined by PCRs targeting *terZ*, *terA*, *terB*, *terC*, *terD*, *terE*, and *terF* (19); present, all genes investigated were present; absent, all genes investigated were absent.
[f] Tellurite resistance (Te^r): EHEC O157:H7 versus SF EHEC O157:NM, $P < 0.000001$ by the χ^2 test.
[g] Presence of *ter* genes: EHEC O157:H7 versus SF EHEC O157:NM, $P < 0.000001$ by the χ^2 test.
[h] Two of three Te^r strains; the third was negative for *ter* genes.
[i] 83 Te^s strains and 1 Te^r strain.

Two of three Te^r SF EHEC O157:NM isolates (3226/98 and 3323/98) had Te MICs of 128 μg/ml, grew well on CT-SMAC and LB-Te agar, and contained all *ter* genes; the remaining Te^r (MIC, 256 μg/ml) SF EHEC O157:NM strain (4180/97) had no *ter* genes (Table 1).

Southern hybridization. *terC* was carried on a ca. 9-kb DNA fragment in *ter*$^+$ SF EHEC O157:NM strains 3226/98 and 3323/98 (Fig. 1, lanes 6 and 7), on a ca. 6.3-kb DNA fragment in strain EDL933 (lane 1), and on no DNA fragments in strains 5288/91, 4180/97, and *ter*-negative SF EHEC O157:NM strain 493/89 (lanes 3 to 5).

Effect of Te^r on detection of EHEC strains. Our study provides for the first time a basis for the inability of SF EHEC O157:NM to grow on CT-SMAC (11), which was until now only speculated to be caused by their Te^s (12, 16). In contrast to EHEC O157:H7, almost all SF EHEC O157:NM strains lack *ter* genes and are Te^s. Low Te MICs for SF EHEC O157 strains and the comparable growth inhibition of these isolates on CT-SMAC and LB-Te agar suggest that Te, and not cefixime, is the growth-inhibiting component in CT-SMAC. The Te^r and Te^s correlated with the presence and absence, respectively, of *ter* genes in all but one of the 153 EHEC O157 strains investigated. The single Te^r, *ter*-negative SF EHEC O157:NM strain (4180/97) is currently being investigated for other presently known mechanisms of Te^r (18). Interestingly, Southern hybridization suggests that the genomic positions of *terC* differ in the two *ter*$^+$ SF EHEC O157:NM isolates and EHEC O157:H7 strain EDL933 (Fig. 1). Studies are under way to determine if the *ter* genes in these SF EHEC O157:NM strains are clustered, similar to those in EDL933 (14), and to determine the genomic location of the *ter* cluster as well as its copy number. Also, further studies should clarify the reasons for the substantially lower frequency of Te^s found among central European EHEC O157:H7 isolates (1.5%) than among North American *E. coli* O157:H7 (20%) (19). Taken together, our data demonstrate a significant difference between EHEC O157:H7 and SF EHEC O157:NM in the frequency of Te^r and *ter* genes, demonstrate a diversity among SF EHEC O157:NM strains as far as the presence of *ter* genes is concerned, and suggest that other mechanisms of Te^r exist in a minority of such strains. However, most importantly, our data clearly indicate that, because of their Te^s, most SF EHEC O157:NM strains are missed by strategies currently used for the isolation of EHEC O157:H7 strains in many clinical laboratories. A similar observation has been reported for a subset of other Te^s EHEC strains (5). The selectivity offered by incorporating Te into agar media, while appropriate for isolating *E. coli* O157:H7 (the most important EHEC serotype worldwide), hinders the assessment of the geographic distribution, medical significance, and epidemiology of SF EHEC O157:NM strains, which in Germany are the second most common cause of HUS (6). Detection opportunities that do not select against SF EHEC O157:NM and, optimally, specifically target these organisms (7) are needed to answer the question about the relative significance of both EHEC O157 pathogens in human diseases.

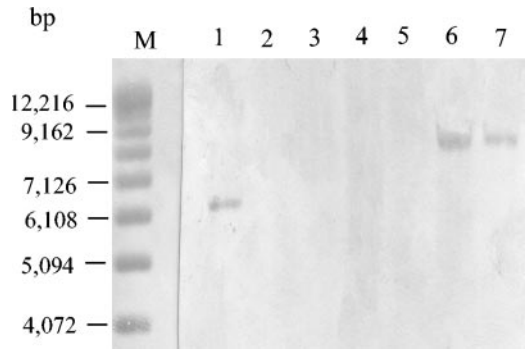

FIG. 1. Hybridization of BamHI-PstI-digested genomic DNA from EHEC O157 strains and controls with the *terC* probe. M, molecular weight marker (1-kb DNA ladder; Gibco-BRL). In lanes 1 to 7, the following strains are displayed (serotype, Te-MIC in micrograms per milliliter, and the presence of *ter* genes as detected by PCR are given in parentheses): lane 1, EDL933 (O157:H7, 128, *ter*$^+$); lane 2, C600 (not available; <1; *ter* negative) (negative control); lane 3, 5288/91 (O157:H7; <1; *ter* negative); lane 4, 4180/97 (SF O157:NM; 256; *ter* negative); lane 5, 493/89 (SF O157:NM; <1; *ter* negative); lane 6, 3226/98 (SF O157:NM; 128; *ter*$^+$); lane 7, 3323/98 (SF O157:NM; 128; *ter*$^+$). Two *terC* copies detected in EDL933 after DNA separation by pulsed-field gel electrophoresis (19) were not distinguishable by conventional gel electrophoresis.

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